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Multiple-Paper Chromatogram

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A method was required by which mixtures of organic acids or alkaloids in semimicroquantities could be resolved into their pure components to yield sufficient amounts for the preparation of derivatives for identification. A new apparatus, the "Chromatopack," consists of a pack of Whatman No. 1 filter paper strips compressed between two stainless steel plates. The zones obtained from mixtures of pure compounds were separated and yielded up to 10 mg. of each constituent. The method lends itself to easy detection of zone positions with resolution equal to that obtained with single sheets of paper but producing semimicro quantities of pure materials. This extends the usefulness of paper chromatography from a submicroqualitative tool to a semimicropreparative tool.

THE usefulness of the papergram technique in the resolution of mixtures of many kinds of materials has been established. The original technique of Consden *et al.* (*1*) has been modified in order to handle smaller and smaller amounts of materials (*6*). However, for preparative work and for identification experiments, where known materials are not available, these procedures are not satisfactory and modifications such as the "Chromatopile" (*3, 4*) have been adopted. In this laboratory the Chromatopile has proved somewhat inconvenient because of the formation of zones shaped like "inverted cones," which made it difficult to separate the fractions, and the operation of detecting the zones by removing many sheets from the pile was relatively tedious. As a result, a new technique has been developed which requires the simplest apparatus and lends itself to easy detection of the zones and to their ultimate isolation and elution. The setup has been termed a "Chromatopack."

A photograph of the Chromatopack is shown in Figure 1.

It consists of a pack of long strips of Whatman No. 1 paper (18 × 2 inches or wider) clamped between two stainless steel plates. The strips are cut from the usual 18.25 × 22.5 inch sheets by means of a paper cutter. For use, the sample is placed on a line 2.0 cm. from one end of each of 100 or more strips, using about 0.01 ml. of solution per centimeter of width. A No. 26 hypodermic needle and a 0.5-ml. syringe with the plunger removed are used to distribute the same solution. Ten blank strips are placed on each side of the pack of sample strips. The entire pack is carefully aligned and placed between the stainless steel plates, so that the end on which the sample was deposited is about 5 mm. from the end of the plates. After tightening the nuts to compress the sheets, the entire assembly is placed upright in a 12 by 24 inch (30 × 60 cm.) glass cylinder with the sample end resting in a stainless steel tray. The solvent, either the organic phase of the equilibrated two-phase solvent or a one-phase solvent containing water, is carefully added to the tray and the chromatogram is developed until the solvent front has moved to just below the top of the pack. At this time, the pack is removed, the plates are loosened, and the filter paper pack is withdrawn. A sheet from each side and from the center of the pack is removed, dried, and sprayed for detection of the bands. The pack is cut and the different zones are isolated. These fractions are eluted and subjected to qualitative analysis. No quantitative analyses have been carried out to date, but several investigations are in progress.

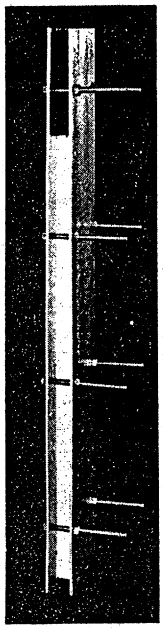

Figure 1. Filter Paper Chromatopack after Removal from Solvent Chamber

EXPERIMENTAL

The technique was tested using dyes, nonvolatile organic acids, and tobacco alkaloids. Chromatopacks consisting of 200 strips (18 × 2 inches), each containing the solute from 0.05 ml. of sample solution, were assembled, placed in the glass cylinder, and developed until the solvent front had moved in excess of 28 cm. At this time the pack was disassembled and the test sheets were removed, dried, and sprayed for detection of the zones.

The individual components of the mixtures, their concentration, range of spot movement, and their R_F values, as well as the solvent and spray materials, are summarized in Table I.

DISCUSSION

The procedure of placing the sample on the separate sheets is somewhat tedious, but it can be eased by placing the sample on the large sheets of paper (18.25 × 22.5 inches) prior to cutting the strips. If precut strips are available, a microburet having a mechanical delivery attachment can be used. The relative inefficiency of this step is more than overcome by the ease of assembly, detection of zone position, and isolation of the pure components which have been resolved from the mixture.

In the preliminary experiments with the organic acids,

Table I. Summary of Experimental Results Obtained Using Chromatopack

Type of Mixture	Components of Mixtures	Concentration *Mg./ml.*	Range of Spots *Cm.*	Solvent Front *Cm.*	R_F	Solvent Composition *Parts or ml.*	Spray Mixture
Dyes	Fuchsin G	5	0.3-1.8	28	0.04	1-Butanol, 40	None needed
	Methylene blue	5	10.6-12.4		0.41	Abs. ethyl alcohol, 10	
	Crystal violet	5	22.9-28.0		0.91	Water, 50	
Organic acids	Tartaric	5	11.0-14.5	33.1	0.40	5 *M* formic acid, 50	Bromophenol blue
	Malic	15	16.9-18.7		0.53	1-Pentanol, 50	
	Succinic	15	20.9-24.3		0.68	Addnl. abs. ethyl alcohol to form one phase	
Tobacco alkaloids	Nornicotine	5	0.3-4.0	34.0	0.06	1-Butanol, 85	Iodine
	Nicotine	5	7.5-19.2		0.36	Benzene, 5	
	Nicotyrine	5	21.5-31.1		0.80	Buffer[a], 30	

[a] Mixture of 9.5 ml. of 0.2 *M* acetic acid, and 90.5 ml. of 0.2 *M* sodium acetate; pH, 5.6 (*5*)

the organic phase of the two-phase system of Lugg and Overell (*2*) contained insufficient formic acid to suppress the ionization of the organic acids and resulted in tailing and poor resolution. Addition of enough ethyl alcohol to cause the formation of a single-phase system produced the results shown in Table I.

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